

# Uptake, Translocation and Subcellular Distribution of Three Triazole Pesticides in Rice

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## Research Article

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# Abstract

Triazole pesticide is a widely-used pesticide for the control of pathogenic fungi in crops and its extensive use has caused food safety issues due to its uptake by edible crops. The residue of triazole pesticides has caused many toxicity risks and food safety problems. In this study, the uptake, translocation, and subcellular distribution of three triazole pesticides (triadimefon, tebuconazole, and epoxiconazole) in rice were investigated. The results showed that the three selected triazole pesticides could be taken up by rice roots, but their distribution in plant tissues was different. The accumulation of the three pesticides in rice root followed the order of epoxiconazole > tebuconazole > triadimefon, while a reversed order was observed in rice shoots. The movement of triazole pesticides within rice tissues involved both symplast and apoplast pathways, with triadimefon preferentially through the symplast pathway and epoxiconazole through the symplast pathway. The subcellular distribution reveals that all pesticides have a higher proportion in cell walls than in cell organelles and soluble components. Epoxiconazole has the highest accumulated capacity in the cell wall (45%-67%) and triadimefon was more concentrated in the soluble components (24%-29%). However, there exists no significant difference in the amount of three pesticides in cell organelles. The pesticide uptake, the movement of pesticides in symplast and apoplast, and the subcellular distribution of the three pesticides are related to the hydrophobicity of the three pesticides.

## 1 Introduction

Pesticide contamination in the environment and food is one of the most serious problems in global agriculture. The annual global consumption of pesticides is 3.5 million tons, with China as the largest consumer (Pretty and Bharucha, 2015; Pan et al., 2019). Although pesticides play an important role in the management of plant disease during agricultural production, extensive and excessive application of chemical pesticides has caused serious environmental and ecological problems (Oerke, 2006; Kvesitadze, 2015; Taiwo, 2019). Pesticide residues can accumulate in the water, soil (Lamers et al., 2011; Van Toan et al., 2013; Chau et al., 2015), fields, and ditches, adversely affecting target and other organisms (Dey et al., 2013; Sruthi et al., 2016). Crops can take up pesticide residues and translocate them to edible structures, causing food-safety issues (Kvesitadze et al., 2006).

Triazole pesticides are a class of broad-spectrum fungicides marked by their low-toxicity and high-efficiency (Bakanov et al., 2020). Triazole fungicides can affect cell membrane formation and inhibit fungal growth by inhibiting the biosynthesis of steroids and ergosterols in fungal cells. Some triazole fungicides also have the function of regulating physiological efficiency, changing the binding group, insecticidal and herbicidal activities (Buchenauer, 1987). Triazole pesticides are widely used in crops because of their high absorbability and persistence. It is an emerging organic pollutant of concern (Angioni 2003; Elskus and Hackley, 2012). Triazole pesticides are widely applied during the production of food crops and also used as medicinal materials, consequently leading to the wide-spread distribution of pesticide residues in the environment (Julio, et al., 2020). Some studies have demonstrated that triadimefon has toxic effects on zebrafish and xenopus, like hormone dyscrasia and reproductive problems (Ma et al., 2020, Zhang et al., 2020). Moreover, the metabolites of some pesticides, such as

triadimefon and triadimenol, can induce cytotoxicity in human beings (Xu *at al.*, 2020). Epoxiconazole is an inhibitor of 11  $\beta$ -hydroxylase and aldosterone synthase, and prolonged exposure can cause cardio-metabolic diseases, chronic kidney disease, mass, and immune-related disorders (Akram et al., 2019). The residues of triazole pesticides and the law of residues in food plants have become an urgent problem to be solved, and their uptake, transport, and distribution in rice have not been fully investigated.

In the present study, the uptake, translocation, and subcellular distribution of three widely-used triazole pesticides (triadimefon, tebuconazole, and epoxiconazole) in rice seedlings were investigated under hydroponic conditions. Root concentration factor (RCF) and translocation factor (TF) were used to compare the difference among the uptake and accumulation behavior of three pesticides in rice plants. The proportion of the three pesticides within rice tissues in symplastic and apoplastic pathways was calculated. Similarly, the proportion of the three pesticides in cell walls, water-soluble components, and cell organelles was computed.

## 2 Materials And Methods

### 2.1. Chemical Reagents and Laboratory Materials

Standards of the three selected pesticides, triadimefon, tebuconazole, and epoxiconazole, all with a purity of > 99%, were purchased from Aladdin Reagent Corporation (Shanghai, China). The physicochemical properties of these pesticides are shown in Table 1. Acetonitrile (HPLC grade) was purchased from Merck Company (Germany). Anhydrous magnesium sulfate (MgSO<sub>4</sub>), sodium chloride (NaCl), sodium hypochlorite and alcohol (98%) were purchased from Sinopharm Group Co. Ltd. (Shanghai, China), primary secondary amine sorbent (PSA), and graphitized carbon black (GCB) were purchased from Shanghai Anpel Scientific Instrument Company (Shanghai, China). Tris-HCl pH 6.5/7.5,  $\beta$ -mercaptoethanol (BME, C<sub>2</sub>H<sub>6</sub>OS), sucrose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>), ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>), dithiothreitol (DTT, C<sub>4</sub>H<sub>10</sub>O<sub>2</sub>S<sub>2</sub>), and Yoshida rice nutrient solution were purchased from Beijing Coolaber Technology Co. Ltd (Beijing, China).

Table 1  
Selected physicochemical properties of pesticides used in this study.

Pesticide	Formula	Molecular weight	Solubility(mg/L)	logK <sub>ow</sub>
Triadimefon	C <sub>14</sub> H <sub>16</sub> ClN <sub>3</sub> O	293.7	72	2.77
Tebuconazole	C <sub>16</sub> H <sub>22</sub> ClN <sub>3</sub> O	307.8	36	3.11
Epoxiconazole	C <sub>17</sub> H <sub>13</sub> ClFN <sub>3</sub> O	329.8	6.63	3.44

### 2.2. Plant Culture and Exposure Experiments

Seeds of rice (*Oryza sativa* L. *japonica*. cv. *Nipponbare*) were provided by Jiangsu Academy of Agricultural Sciences. Rice seeds were soaked in 3% sodium hypochlorite solution and then 75% alcohol solution for surface disinfection. Following a thorough rinse with deionized water, the seeds were placed on water-wet filter paper in a petri dish and kept under 30 °C in darkness for germination. Rice seedlings with uniform size were selected and transplanted to black plastic hydroponic containers (9cm×13cm×12cm) and cultured under controlled conditions (light intensity  $130 \pm 5 \mu\text{mol}/\text{m}^2\text{s}$ , photocycle 16 h light / 8 h darkness, relative humidity 60%, day/night temperature 32/25 °C). After the rice had grown three intact leaves, healthy seedlings with uniform size were selected for subsequent exposure tests.

Rice seedlings were exposed to 1 mg/L of a pesticide (triadimefon, tebuconazole, or epoxiconazole) by spiking a calculated quantity amount of the pesticide standard into the nutrient solution for plant culture. All the experiments were set in triplicate. At time intervals of 0, 3, 6, 12, 24, and 48 h, rice seedlings and nutrient solutions were sampled for further analysis. Nine rice plants were selected from each hydroponics box, washed with sterile water and dried, and stored at 20 °C before analysis.

### 2.3 Analysis of pesticide residues in symplast and apoplast of the plant

The separation of plant symplast and apoplast was carried out by vacuum osmosis centrifugation (Li et al., 2018a, Li et al., 2018b). Rice seedlings were cut into shoot and root after careful rinse with distilled water to remove pesticide residues sorbed on the plant surface. Plant root or shoot samples were weighed into a 10mL centrifuge tube and mixed with 5ml buffer (50mmol/L Tris-HCl pH 8.0, 0.6 mol/L NaCl, 0.1%  $\beta$ -mercaptoethanol). The tubes were transferred to a vacuum dryer under 70 kPa for 10min. The samples were taken out and drained and put into a 5mL syringe. Then, the syringe was placed in a centrifuge tube of 10mL at 4°C and centrifuged at 5000 r/min for 10min to collect extracellular fluid. The same separation procedure was performed 3 times. The extracted symplast and apoplast were weighed and extracted with the pesticide extraction method in 2.5.

### 2.4 Analysis of pesticide residues in subcellular components

Rice roots and shoots were rinsed to remove the pesticide, wipe off the water, put it into 10mL centrifuge tubes, and put three steel balls into it. The samples were quickly frozen in liquid nitrogen and then ground into powder using a grinder. Then added a pre-cooled buffer (50mM Tris-HCl pH 7.5, 250mM sucrose, 5.0 mM ascorbic acid, 1.0 mM dithiothreitol  $\text{C}_4\text{H}_{10}\text{O}_2\text{S}_2$ ) to the homogenate and placed in the ice bath. The volume ratio of the sample size to buffer liquid was 1:10. Separated cell walls, organelles, and soluble components were by differential centrifugation. The cell wall was precipitated at 4°C and 300g centrifugation for 30s. The supernatant was transferred to a 10mL centrifuge tube and centrifuged at 15000g for 45min. The sediments were organelles and the supernatant were soluble components of cells (cell matrix and vacuoles). Each cell component shall be extracted according to the pesticide extraction method in 2.5.

### 2.5 Pesticide analysis

First, 0.2g of rice roots or shoots were put into a 10mL centrifuge tube, add two steel balls and 2ml acetonitrile, and ground in a grinder (Thmorgan, China Beijing) for 20 min. Then add 0.5g NaCl to the centrifuge tube, vortex for 30s, centrifugation at 5000 rpm for 5min, and 1ml supernatant was taken into the new centrifuge tube. 75mg anhydrous  $\text{MgSO}_4$ , 15mg PSA (root), 75mg anhydrous  $\text{MgSO}_4$ , 15mg PSA, and 10mg GCB (shoot) were added into the supernatant respectively. The tube was rotated for 30s and centrifuged at 5000rpm for 1min. The supernatant was filtered through a 0.22  $\mu\text{m}$  organic phase membrane in a vial and tested by LC ~ MS. 1 mL nutrient solution, 4 mL acetonitrile, 1g NaCl, and 200mg anhydrous  $\text{MgSO}_4$  were placed in a 10mL centrifuge tube and shaken in the grinder for 20min. Then 1mL supernatant was filtered through a 0.22  $\mu\text{m}$  organic phase membrane in vials and tested by LC-MS.

All samples were analyzed by Agilent 1260/6410 triple quadrupole mass spectrometer connected with a positive electrospray ionization source (HPLC-MS/MS) (Agilent Technologies, USA). Chromatographic separation was achieved by using a ZORBAX SB-C18 column (4.6 mm  $\times$  250 mm, 5  $\mu\text{m}$ ) at 30°C. The mobile phase was 10% acetonitrile aqueous solution, the flow rate was 0.4 mL/min, and the injection volume was 5  $\mu\text{L}$ . The ionspray source of the mass spectrometer adopts the positive ion mode. The ionspray voltage is 4000 V, the temperature is 300°C, and the colliding gas is helium. Multiple response monitoring (MRM) model was used for quantitative analysis. To evaluate the effects of three pesticides on the normal growth of rice seedlings. The rice samples to be tested were weighed at 0h, 3h, 6h, 12h, 24h and 48h in the pesticide treatment group and the blank control group, respectively, to analyze the change of fresh weight of rice. Recorded the loss of solution within 0 ~ 48h and calculated the transpiration rate of rice plant.

The recoveries of the three pesticides in rice root, shoot, and solution are shown in Table S1. The blank samples (root, liquid, and water) were configured at standard concentrations of 0.05, 0.5, and 1mg/kg. Extract and purify the pesticide using the same method as described above. The result shows that the recovery rates of the three pesticides in shoot, root, and solution were 73 % ~ 116%, 78% ~ 102% and 75% ~ 90%, respectively (Table S1).

## 2.5. Data analysis

Root concentration factor (RCF) is calculated as the concentration ratio of a pesticide in plant roots to that in the nutrient solution for plant culture. (Li et al., 2018a). The translocation factor (TF) is calculated as the concentration ratio of a pesticide in plant shoot to that in plant root (Wu et al., 2018). The RCF and TF can be calculated using the following equations:

$$RCF = C_{\text{root}} / C_{\text{solution}} \quad (1)$$

$$TF = C_{\text{root}} / C_{\text{shoot}} \quad (2)$$

Where  $C_{\text{root}}$  (mg/kg),  $C_{\text{shoot}}$  (mg/kg) and  $C_{\text{solution}}$  (mg/L) are the concentrations of a pesticide in plant root, shoot, and nutrient solution, respectively.

To evaluate the effect of three pesticides on the transpiration of rice plants, the average transpiration rate of rice plants within 48h was calculated using the following equation:

$$\text{Transpiration rate (mL/g/ h)} = \frac{V(0h) - V(48h) - V(\text{sampling}) - V(\text{evaporation})}{M(\text{rice}) \times T} \quad (3)$$

$V(0h)$  is the volume of solutions at 0h;  $V(48h)$  is the volume of solutions at 48h;  $V(\text{sampling})$  is the total volume sampled between 0 and 48h;  $V(\text{evaporation})$  is the volume of natural evaporation of the solution from 0 to 48 h without rice plants.  $M(\text{rice})$  is the total mass of a box of rice.  $T$  is 48 hours.

SPSS 20 (IBM, Armonk, NY, USA) was used for one-way ANOVA.

## 3 Results And Discussion

### 3.1. Uptake of pesticides by rice plants

Many studies have reported that rice roots could take up the selected pesticides and translated them to the shoot. The transpiration rates of rice in the control group and the three pesticide treatment groups were 0.0072, 0.0068, 0.0069, and 0.0064 mL/g/h, respectively, with no significant difference (Fig. S1). Rice seedling growing in pesticide treatment groups was normal compared with that in the blank control group. The concentration of the three pesticides in solution and plant tissues was shown in Fig. 1. In the shoots, the concentration of triadimefon increased to 0.53 mg/kg at 6h, and then decreased slowly to 0.44 mg/kg at 48h (Fig. 1A). The concentrations of tebuconazole and epoxiconazole continuously increased to 0.925 mg/kg and 0.389 mg/kg, respectively, at 48h. Triadimefon had the highest accumulation, but the fastest degradation rate in the shoot. In plant roots, the pesticide residue concentration in the roots increased first and then decreased (Fig. 1B). tebuconazole peaked at 2.73 mg/kg at 12h and then dropped to 2.20 mg/kg at 48h. Triadimefon reached a maximum of 1.43 mg/kg at 6h and then began to decrease to 0.825 mg/kg at 48h. Concentrations of epoxiconazole reached a maximum of 4.26 mg/kg at 24h and then began to decrease, reaching 3.49 mg/kg at 48h. The concentrations of the three pesticides decreased with the increase of exposure time, and the pesticide concentrations in the nutrient solution began to increase from 12h. The final concentrations were 0.881 mg/kg, 1.07 mg/kg and 0.970 mg/kg at 48h (tebuconazole, triadimefon and epoxiconazole). However, the pesticide concentration in the solution decreased first and then increased with the increase of time, which may be due to the decrease of pesticide absorption in plant tissues. With the uptake of pesticides, water also volatilizes with transpiration. When the rate of water transpiration is higher than the absorption rate of pesticides, the concentration of pesticides in the solution begins to rise.

Table 2 and Fig. 2 show the root concentration factor (RCF) and translocation factor (TF) of three pesticides in rice plants. The RCF was used to describe the ability of rice roots to take up a compound from solutions (Wu et al., 2013). The average RCFs of tebuconazole, triadimefon, and epoxiconazole were 3.08, 1.27, and 4.37, respectively. Epoxiconazole has the highest average RCF, followed by tebuconazole and triadimefon. The result of RCFs reveals that tebuconazole was most readily accumulated in plant

roots, and triadimefon was the weakest. TF was used to describe the ability of rice roots to transfer compounds to rice shoots (Wang et al., 2011). The average TF of tebuconazole, triadimefon and epoxiconazole were 0.18, 0.38, and 0.05, respectively. triadimefon has the highest average TF, followed by tebuconazole and epoxiconazole. all TFs were smaller than one, which reveals that the three pesticides were more easily accumulated in plant roots and less transported in plant shoots. Among the pesticides, triadimefon was the most readily accumulated in plant shoots, and tebuconazole was the weakest. The order of the three pesticides in TF was the opposite of the order in RCF. RCF is positively correlated with  $\log K_{ow}$  (Table 1) of pesticides and negatively correlated with water solubility (Table 1). Epoxiconazole has the lowest water solubility and the strongest hydrophobicity and is most easily absorbed and accumulated by plant roots. This might be the possible the reason why the RCF value of epoxiconazole is the largest in rice plants. TF was used to describe the ability of rice roots to transfer compounds to rice shoots (Wang et al., 2011). The average TF of tebuconazole, triadimefon and epoxiconazole were 0.18, 0.38, and 0.05, respectively. TFs is negatively correlated with  $\log K_{ow}$  of pesticide and positively correlated with water solubility (Wu et al., 2018). Triadimefon has the highest water solubility and the weakest hydrophobicity and is most easily transferred from root to shoot.

Table 2  
Root concentration factor (RCF) and transfer factor (TF) in rice.

Exposure Time (h)	RCF			TF		
	Teb <sup>a</sup>	Tri <sup>b</sup>	Epo <sup>c</sup>	Teb <sup>a</sup>	Tri <sup>b</sup>	Epo <sup>c</sup>
3	2.69	0.89	3.51	0.06	0.36	0.02
6	3.15	1.63	4.86	0.11	0.35	0.04
12	3.75	1.64	5.20	0.13	0.35	0.05
24	3.29	1.41	4.69	0.15	0.35	0.05
48	2.49	0.77	3.60	0.42	0.49	0.11
Average	3.08	1.27	4.37	0.18	0.38	0.05
<sup>a</sup> , tebuconazol; <sup>b</sup> , triadimefon; <sup>c</sup> , epoxiconazole						

Many previous studies reported that the uptake and translocation of pesticides wererelated totheir physicochemical properties. As shown in Table 1 epoxiconazole has the lowest water solubility and the strongest hydrophobicity. On the contrary, triadimefon has the highest water solubility and the weakest hydrophobicity. The ability of plant roots to absorb non-ionic compounds is influenced by the chemical properties of the compounds (Collins et al., 2006; Li et al., 2019). This is consistent with theabove experimental results. organic compounds with larger  $\log K_{ow}$  are easy to be absorbed by plant roots, because of their strong hydrophobicity, they are easier to be absorbed by roots and concentrated in lipid membranes. Less hydrophobic organic compounds are less concentrated in roots and can enter plants

and transmit to other locations with transpiration streams (Létondor et al., 2015). After entering the root system, compounds would conduct conduction to the upper part of the plant with transpiration, and triadimefon with higher water solubility would be more easily conducted from the root of the plant and accumulated in the shoots of rice (Pullagurala, 2018). It can be inferred that the combination of multiple physical and chemical factors determines the transfer of pesticides from the external medium to the rice root and other locations.

### 3.2. Distribution of pesticides in symplast and apoplast

Figure 3 shows that the three pesticides distributed in the symplast and apoplast of root and shoot of rice. The proportions of triadimefon, tebuconazole and epoxiconazole in the symplast and apoplast of rice plants were 15% – 33%, 6% – 31%, 7% – 37% and 67% – 85%, 69% – 94%, 63% – 93%, respectively. The distribution of the three pesticides in the symplast was significantly larger than that in the apoplast. In general, triadimefon, tebuconazole, and epoxiconazole were mainly accumulated in the symplast after entering the plant, indicating that all the three pesticides had strong transmembrane transfer capacity. The proportion of tebuconazole and triadimefon pesticides accumulated gradually in shoot symplast. The accumulation of tebuconazole in symplast of shoot increased at 12 and 24 h. The accumulation of triadimefon in shoots, epoxiconazole in roots and shoots showed little change in apoplast and symplast. In the root, the mean values of the three pesticides accumulated in the symplast and apoplast were 74%, 74%, 66%, and 26%, 26%, and 36% respectively. In the symplast, the proportion of triadimefon and tebuconazole was same, while the proportion of epoxiconazole was largest, and the order was opposite in the apoplast. The results showed that triadimefon and tebuconazole were more readily accumulated in the symplast and epoxiconazole was more accumulated in the apoplast. In the shoot, the mean values of the three pesticides accumulated in the symplast and apoplast were 74%, 91%, 93%, and 26%, 9%, 7%, respectively. The proportion in the symplast followed the order of epoxiconazole > tebuconazole > triadimefon, and the order was opposite in the apoplast. The results showed that epoxiconazole in the shoot was more readily accumulated in the symplast and triadimefon and tebuconazole were more readily accumulated in the apoplast. The accumulation of tebuconazole and epoxiconazole in shoot and apoplast was significantly larger than that in root apoplast, while the accumulation of triadimefon was not significantly different.

Almost all nonionic organic pollutants enter plants by passive absorption, and their migration is driven by transpiration pull (Kvesitadze et al., 2006). Very few organic pollutants with hormonal properties (Phenoxy acid herbicide) can be actively absorbed by plants (Saunders et al., 1965; Devlin *et al.*, 2006). Triadimefon, tebuconazole, and epoxiconazole can be transferred through the apoplast pathway from rice root to other positions by transpiration pull. A large amount of accumulation was found in the rice symplast, indicating that these three pesticides may be actively transported into the cell through the channel proteins on the cell membrane, or only accumulated in the lipid membrane on the cell surface. Besides, the relative proportion of apoplastic and symplastic movement of chemical compounds was also related to the physicochemical properties of pesticides and physiological characteristics of plant tissues (Zhan *et al.*, 2018). It has been demonstrated that organics with small  $\log K_{ow}$  (large

solubility) are easy to be transported through the symplast pathway of plants, while organics with large  $\log K_{ow}$  (small solubility) are easy to be transported through the apoplast pathway (Li *et al.*, 2020). Epoxiconazole is the most hydrophobic of the three pesticides, its  $\log K_{ow}$  is 3.44 and water solubility is only 6.63mg/L. Tebuconazole and epoxiconazole had strong hydrophobicity and  $\log K_{ow}$  were 2.77 and 2.77. Our results showed that the larger the pesticide  $\log K_{ow}$  was, the easier to accumulate in the symplast in the shoot, while the smaller the  $\log K_{ow}$  was, the more likely it was to accumulate in the apoplast in the shoot. But this order is not significant in the root.

### 3.3. Subcellular distribution of pesticides in rice tissues

Figure 4 shows the proportions of triadimefon, tebuconazole, and epoxiconazole in the subcellular distribution in roots and shoots. The proportions of triadimefon, tebuconazole and epoxiconazole in the soluble components, organelle and cell wall of rice plants were 24% – 29%, 14% – 26%, 11% – 21%; 22% – 29%, 15% – 26%, 12% – 39% and 41% – 54%, 52% – 72%, and 45% – 77%, respectively. The distribution of the three pesticides in the cell wall was significantly larger than that in the soluble components and organelle. Triadimefon, tebuconazole, and epoxiconazole were mainly accumulated in the cell walls after entering the plant. The proportion of tebuconazole and epoxiconazole pesticides accumulated gradually in shoot cell wall. The proportion of triadimefon in shoot cell wall and the three pesticides in root cell wall showed little change. The proportion of tebuconazole and epoxiconazole pesticides accumulated decreased gradually in shoot soluble components. The proportion of triadimefon in shoot soluble components and the three pesticides in root soluble components showed little change. The proportion of the three pesticides in root and shoot organelles showed little change. In the root, the mean values of the three pesticides accumulated in the soluble components, organelle, and cell wall were 28%, 20%, 14%; 28%, 18%, 22% and 44%, 62%, 63%, respectively. In the shoot, the mean values of the three pesticides accumulated in the soluble components, organelle, and cell wall were 26%, 16%, 13%; 24%, 20%, 18%, and 50%, 64%, 69%, respectively. It is showed that tebuconazole is more easily accumulated in the soluble component and epoxiconazole was more accumulated in the cell wall.

The results were related to the water solubility of the three pesticides. The water solubility of the three pesticides is triadimefon > tebuconazole > epoxiconazole. Wang *et al.* (2019) found that because of its strong water solubility, the insecticide thiamethoxam was more easily dissolved in the cell fluid, and had a higher proportion in the soluble components of the root and bud cells of young cabbage. The proportion of triadimefon in root cell walls was significantly lower than that of tebuconazole and epoxiconazole. Plant cell walls are mainly composed of polysaccharides, structural proteins, lignin, lectins, mineral elements, and lipids. In solid solution systems, organic chemicals with low solubility are more likely to be separated into solid phases with high lipid content (Collins *et al.*, 2006; Li *et al.*, 2005). Triadimefon was more soluble than tebuconazole and epoxiconazole and was not easily retained during root uptake and transfer. Kang *et al.* (2010) found that plant organelles contain more lipid components (15 ~ 30%) than cell walls, which are mainly composed of polysaccharides (90%). In general, more hydrophobic compounds are accumulated in plant tissues with higher lipid content (Gao and Zhu, 2004).

Compared with the proportion of pesticides accumulated in the cell wall, the three pesticides were mainly accumulated in the cell wall. The reason for this may be associated with the ability to cross the membrane into the cell. Tebuconazole and epoxiconazole were mainly distributed on the cell wall in rice shoots and leaves, and they were accumulated in trace amounts in organelles and soluble components. This might be because after the pesticide is absorbed by the root, tebuconazole, and epoxiconazole with strong hydrophobicity are largely accumulated in the solid phase of the root, and only a small amount of pesticides is transmitted to the shoots through the xylem. Moreover, due to hydrophobicity, they are mainly concentrated in the cell wall, and only a few of them are transported into the cell membrane. Studies have found that dinotefuran, thiamethoxam, and imidacloprid are mainly accumulated in the soluble components of cells, which are relatively easy to transfer from roots to stems and then to leaves (Dettenmaier, et al., 2008). The proportion of difenoconazole in solid subcellular components was higher than that in soluble components, resulting in its accumulation in roots and less translocation in stems and leaves (Ge, et al., 2017). In lettuce with a low concentration of PAE (phthalic acid ester) and a low concentration of PFOA (Perfluoro octanoic acid), root cell walls and organelles were the dominant storage compartments for PAEs and PFOA, resulting in less translocation to buds. Ju et al. (2020) found that the transportation of pesticide from root to stem and stem to leaf was adversely affected by subcellular fraction concentration factor (SFCF) of cell walls and organelles.

## 4 Conclusion

Our results reveal that the rice plant could take up triazole pesticide (triadimefon, tebuconazole, and epoxiconazole) from the root and translocate it to the aerial part. The distribution of pesticides within the rice plant was affected by the properties of chemicals, with hydrophobic pesticides tending to retain in plant roots, while highly soluble pesticides preferentially accumulating in plant shoots. Epoxiconazole is the most hydrophobic and is easy to accumulate in the root system of plants. Triadimefon has the highest TF, which can be transferred from the root to the aboveground part in plants, and the degradation ability of triadimefon in plants is stronger than the other two pesticides. Both symplast and apoplast pathways may participate in the uptake and transport of triazole pesticides in rice plants. This indicates that triazole pesticides have good transmembrane transport capacity. The subcellar distribution of pesticides was influenced by the chemical properties of pesticides and the lipid content of plant tissue. The more hydrophobic the pesticides are, the more likely they are to be concentrated in the tissues with higher lipid content, resulting in their difficulty in transfer. Results of this study could help to understand the absorption and transport behavior of triazole pesticides in food crops and provided useful information for the safety risk assessment of agricultural products.

## Declarations

### Ethics approval and consent to participate

(Not applicable)

## Consent for publication

(Not applicable)

## Availability of data and materials

The data are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

Haocong Li: Conceptualization, Methodology, Software, Investigation, Writing - Original Draft.

Yong Li: Resources, Writing - Review & Editing, Supervision, Data Curation.

Wenfeng Wang: Validation, Formal analysis, Visualization, Software.

Qun Wan: Validation, Formal analysis, Visualization, Software.

Xiangyang Yu: Writing - Review & Editing, Supervision.

Wenjing Sun: Resources, Writing - Review & Editing, Supervision, Data Curation.

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# Figures

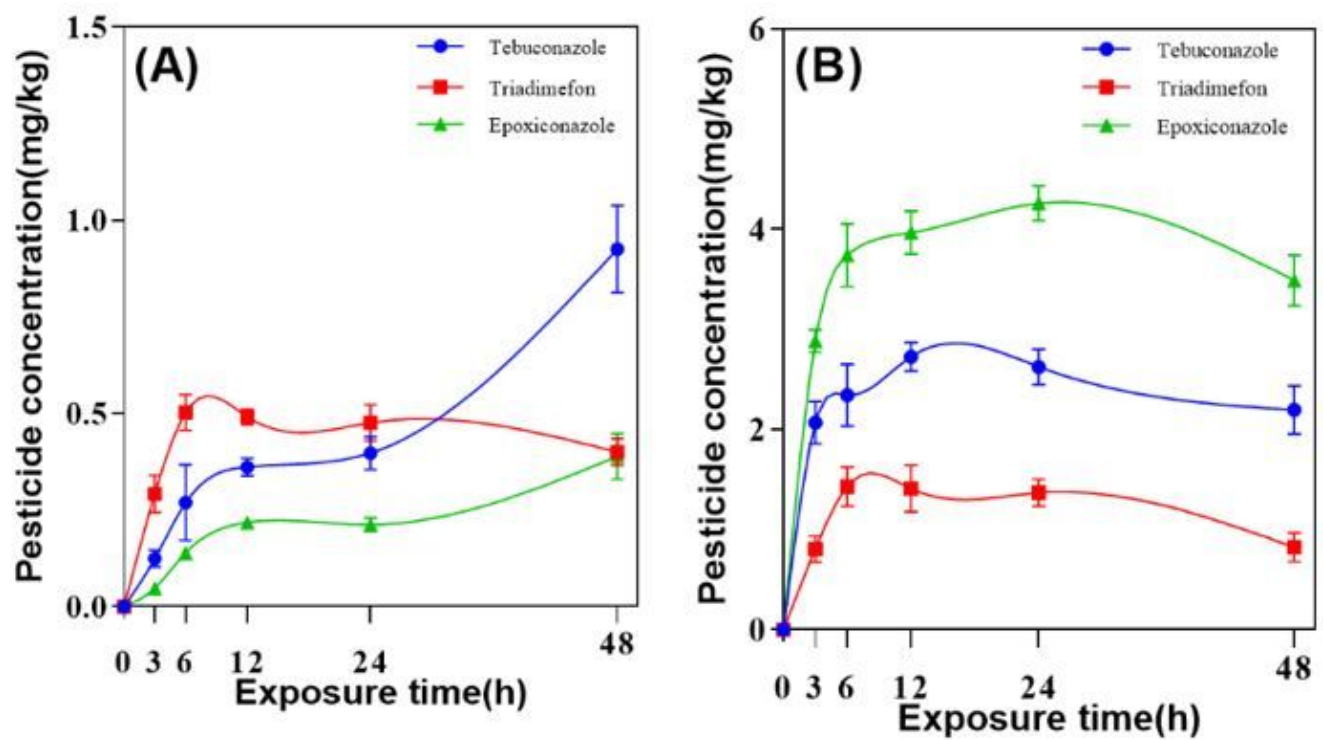


Figure 1

Pesticides concentrations in rice, shoots (B) roots (A) (n=3).

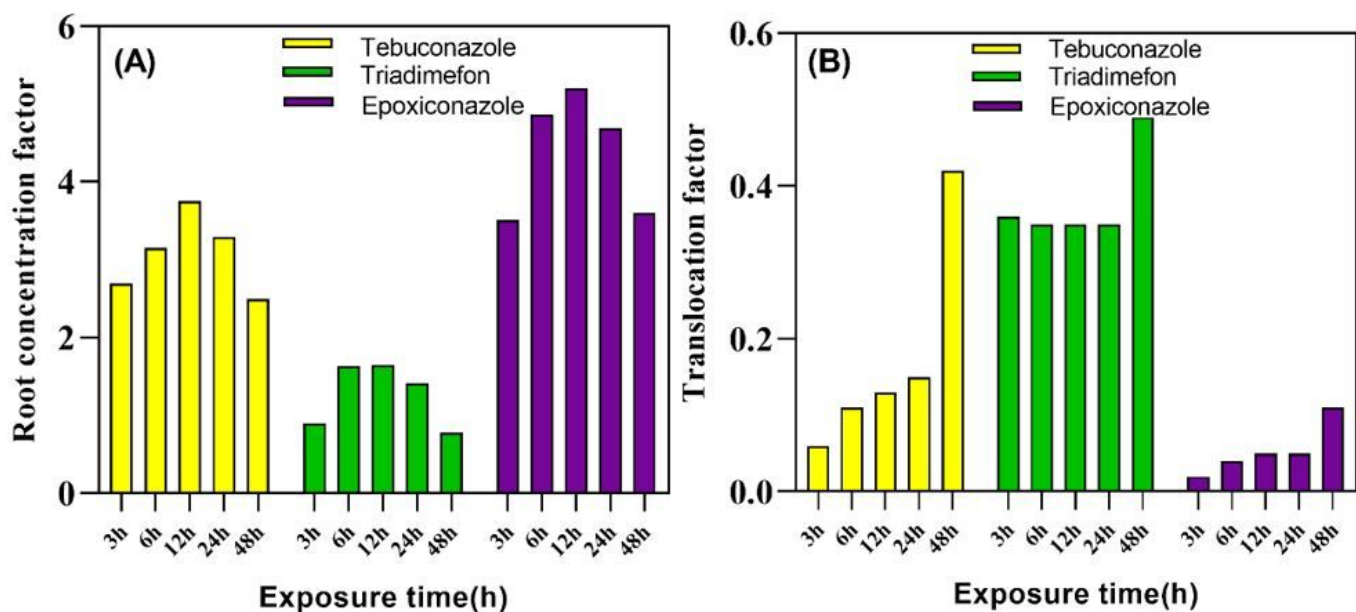


Figure 2

Root concentration factor (A) and transfer factor (B) in rice.

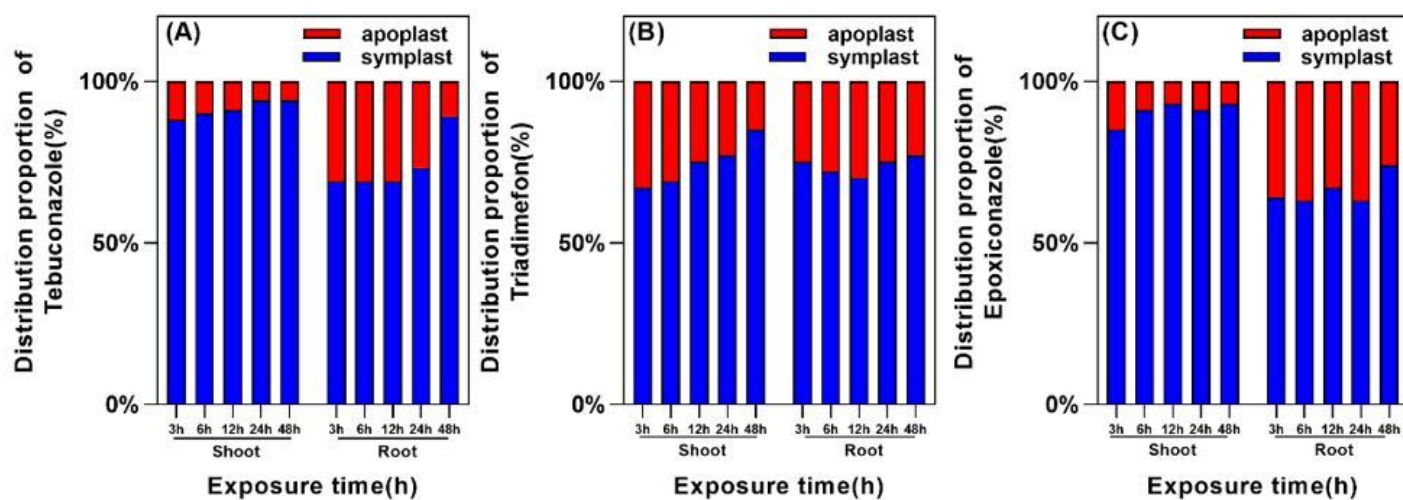
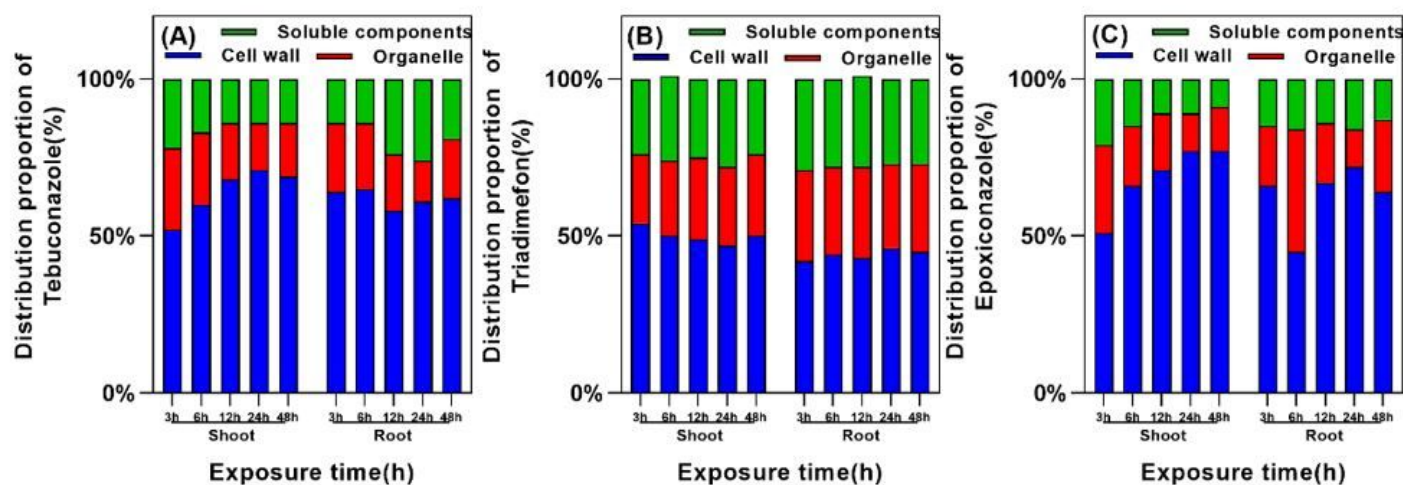


Figure 3

The proportions of pesticides in the symplast and apoplast of rice seedlings (A: tebuconazole; B: triadimefon; C: epoxiconazole; n=3).



**Figure 4**

The proportions of pesticides in the subcellular components of rice seedlings (A: tebuconazole; B: triadimefon; C: epoxiconazole; n=3).

## Supplementary Files

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